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# HYPOSPLENISM

A clinical and experimental study



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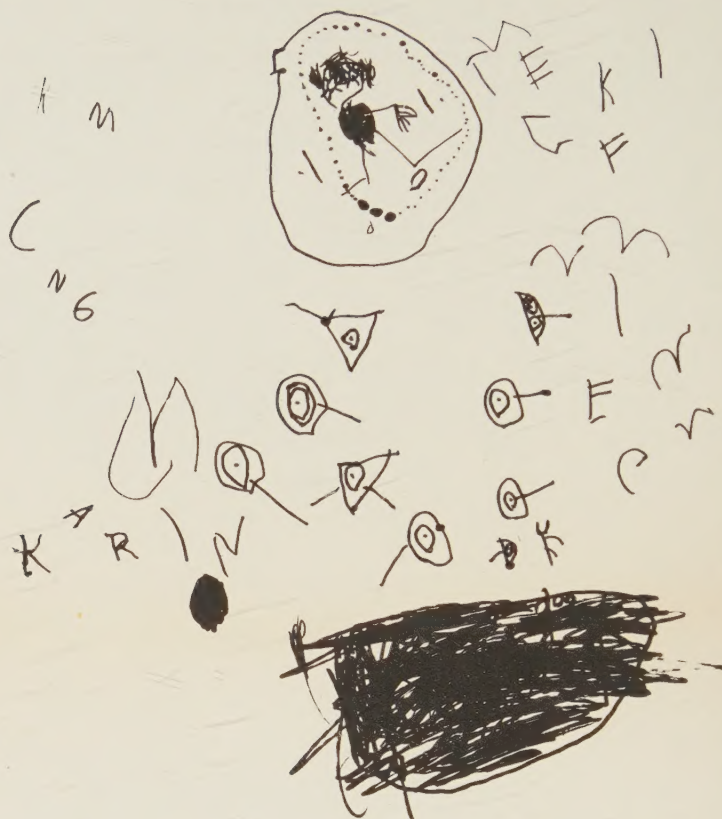
## HYPOSPLENISM

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by

Per Gullstrand

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Splenic regulation of peripheral blood cell elements. Schematic drawing by Karin, 4 years old.





The present thesis is based on the following papers:

- I. Immunocompetence after incidental splenectomy. (In collaboration with H. Graffner and T. Hallberg.) Scand J Haematol (In press).
- II. Improvement of the splenectomized-rat model for overwhelming pneumococcal infection. Standardization of the bacterial inocula. (In collaboration with A. Alwmark, S. Bengmark and C. Schalén.) Eur Surg Res 13:339, 1981.
- III. Overwhelming pneumococcal infection in rats immediately after splenectomy. (In collaboration with A. Alwmark, C Schalén and P. Christensen.) J Surg Res (In press).
- IV. Splenic resection or heterotopic transplantation of splenic tissue as alternatives to splenectomy. Regeneration and protective effect against pneumococcal septicaemia. (In collaboration with A. Alwmark, S. Bengmark, I. Idvall and C.Schalén). Submitted for publication.
- V. Effect of steroids on the outcome of penicillin-treatment in pneumococcal sepsis in splenectomized rats. (In collaboration with A. Alwmark and C. Schalén.) Surgery (In press).

The above articles will be referred to in the text by their respective Roman numerals.

## CONTENTS

### BACKGROUND

|                                                                                |    |
|--------------------------------------------------------------------------------|----|
| Historical notes .....                                                         | 5  |
| Normal splenic functions.....                                                  | 6  |
| Hyposplenism .....                                                             | 7  |
| Overwhelming postsplenectomy infection .....                                   | 7  |
| Hematological changes after splenectomy .....                                  | 9  |
| Methods to preserve splenic function .....                                     | 10 |
| Animal models for the study of overwhelming<br>postsplenectomy infection ..... | 14 |

|                                |    |
|--------------------------------|----|
| AIM OF THE PRESENT STUDY ..... | 16 |
|--------------------------------|----|

### MATERIAL AND METHODS

|                     |    |
|---------------------|----|
| Patients .....      | 17 |
| Animal models ..... | 18 |

### RESULTS AND DISCUSSION

|                                                                                                                                                        |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Immunocompetence after incidental splenectomy in patients<br>undergoing highly selective vagotomy (I) .....                                            | 21 |
| Effect of intravenous injection of <u>Streptococcus pneu-</u><br><u>moniae</u> type 1 in splenectomized rats (II, III) .....                           | 24 |
| Splenic resection and heterotopic autotransplantation of<br>splenic tissue: regeneration and protection against pneu-<br>mococcal challenge (IV) ..... | 28 |
| Treatment of overwhelming pneumococcal infection in<br>splenectomized rats (V) .....                                                                   | 32 |

|                      |    |
|----------------------|----|
| FUTURE ASPECTS ..... | 35 |
|----------------------|----|

|               |    |
|---------------|----|
| SUMMARY ..... | 36 |
|---------------|----|

|                  |    |
|------------------|----|
| REFERENCES ..... | 39 |
|------------------|----|

## BACKGROUND

### Historical notes

More than two thousand years ago, Aristotle concluded that the spleen was not essential to life. This was based on the observation that congenitally asplenic patients had lived apparently normal lives (13).

Galen more conservatively considered it an organ full of mystery (Mysterii pleni organon) and that it probably removed the melancholy of the blood going from the liver to the stomach (102).

A positive statement is made by Pliny, a roman writer and administrator, in his encyclopedic 37-volume "Natural History" (102). According to an old English translation it runs as follows: "This member (the spleen) hath a proprietie by itself sometimes, to hinder a man' s running; whereupon professed runners in the race that bee troubled with the splene, have a devise to burne and waste it with a hot yron. And no marveile; for why? They say that the splene may be taken out of the body by way of incision, and yet the creature live neverthesse; but if it be man or woman that is thus cut for the splene, he or she looseth their laughter by the means. For sure it is that intemperate laughthers have always great spleens." (102).

Though questioned by many historians, the first recorded splenectomy in a human being is claimed to have been performed by Zaccarelli in Naples in 1549. The patient, a 24-year-old woman with splenomegaly, was cured in 24 days (102). From the sixteenth to the beginning of the nineteenth century some reports of successful removal of the spleen exist. These operations most often consisted of exstirpation of the spleen protruding after a penetrating abdominal trauma (27). The first to plan deliberate splenectomy was probably Quittenbaum in 1826, followed by Kuchler in 1855. Unfortunately, both patients died from shock a few hours after the operation. However, improvement in surgical technique and careful selection of patients made splenectomy a preferable procedure in numerous conditions at the beginning of this century (102). Increased understanding of the role of the spleen in hematologic disease rendered it possible for Mayo (1928) to obtain encouraging results on splenectomy in leukemia and lymphoma (116). Today, splenectomy has become an accepted therapy in many hematological disorders, but is still controversial in some (5, 155). Significant changes in the indications for splenectomy have been noted over the years, but since Moynihan (1921) wrote extensive reviews on splenectomy, little has been added to the techniques of splenectomy (126). From being the most common indication for splenectomy, non-surgical injury to the organ today accounts for only about 15 % of all splenectomies (169). In a report from the Ohio State University Hospital, Traetow et al. (1980) found that splenectomy in Hodgkin' s disease had emerged as the most frequent indication



(27 %), followed by incidental splenectomy (20 %) when reviewing the last five years (473 cases) of 30 years of experience (2 417 cases) (169). The indications for splenectomy will not be static. For example the benefit of splenectomy in Hodgkin's disease is under discussion (11,58).

The word "hyposplenism" was established by Dameshek (1955) who set forth criteria for the diagnosis of hyposplenism (47). However, already Eppinger (1913) proposed this term to describe the condition which develops after extirpation of the spleen (64). This condition will be discussed later.

### Normal splenic functions

The spleen represents 25 % of the reticuloendothelial system, reaching its maximum weight at puberty (109). The organ acts like a filter in the blood stream clearing 4 % of the circulating blood volume each minute (63). It also has a regulatory function on circulating blood cell elements, retaining reticulocytes with excessive membrane and weak negative surface charge. Furthermore it removes surface craters and pits from normal red cells and any Howell-Jolly bodies (nuclear remnants), Heinz bodies (denatured hemoglobin), Pappenheimer bodies (iron granules) and acantocytes (spur cells) (45, 62). The role of the spleen in platelet regulation is not fully understood. In the rat, about 15 % of the platelets are pooled in the spleen (85) and it has been suggested that unknown humoral factors of splenic origin exist, which control platelet formation (160). In humans, too, there is an exchangeable splenic platelet pool, which affects the number of circulating platelets (2,103).

The unique splenic circulation has been much debated (22, 101, 112). Using microspheres Chen (1978) demonstrated that 90 % of the blood entering the spleen passes through an "open route", i.e. through splenic sinuses into the red pulp. Thus, the "closed route", where capillary shunts bypass the splenic sinuses, is utilized for 10 % of the blood entering the spleen (33).

The relative importance of the liver and the spleen in the clearance of red blood cells and bacteria from the blood largely depends on the amount of antibodies coating the antigens. In the presence of low levels of specific antibodies, the spleen and not the liver is the main site of sequestration of red blood cells (93, 151).

Antigenic particles entering the spleen first lodge in the red pulp. Within hours, a transport, possibly by mobile macrophages, occurs across the marginal zone into germinal centers where an IgM antibody response is initiated (131). This antibody response, however, is unlikely to affect bacterial clearance from the blood during the early stages of infection with an organism new to the host, but influences the later courses of infection (181).



The spleen also influences on the phagocytes of the blood, i.e. polymorphonuclear leucocytes and monocytes. The motility and phagocytic activity of these cells are stimulated by a leucophilic gammaglobulin termed leucokinin (127,129). This stimulation is probably exerted by a tetrapeptide from leucokinin containing the amino acids threonine, lysine, proline and arginine (128). The tetrapeptide, called tuftsin, is released from its carrier gammaglobulin (leucokinin) by two enzymes; leucokininase, a membrane enzyme with its active site pointing to the extracellular compartment of the phagocyte; and tuftsin endocarboxypeptidase, present exclusively in the spleen (130).

### Hyposplenism

Hyposplenism, as defined by Dameshek (1955), is detected by careful examination of a well-stained, well-spread blood smear which will show Howell-Jolly bodies and target cells (47). This picture is seen not only after splenectomy but also in a variety of other disorders. Hypoplasia of the spleen (97), small spleen in Fanconi's syndrome of hypoplastic bone marrow (70), atrophy of the spleen following thorium dioxide (Thorotrast<sup>R</sup>) administration (19), dermatitis herpetiformis or celiac sprue (30, 67, 115, 141), hemorrhagic thrombocytopenia (114) and thyrotoxicosis (28) are conditions in which hyposplenism may occur. Functional asplenia in sickle cell anemia (138) is probably the most common disease linked with hyposplenism (62). In patients with ulcerative colitis about 40 % will develop hyposplenism during the disease, being severe when pancolitis occurs (150). Finally, infiltration of splenic parenchyma in for example sarcoidosis (81) or amyloid transformation (164) as well as high-dose corticosteroid therapy (14) might impair splenic function.

Already in 1919 Morris and Bullock stated that "it seems possible that anemia and leucocytosis which are temporarily observed (after splenectomy; author's remark) are an indication of increased susceptibility to infection and imperfect reaction against it" (125). At that time, total splenectomy was the only way of reducing a mortality rate of 90 % seen in patients with splenic rupture given non-operative treatment (31). It was not until 1952, when King and Shumacker (99) reported serious infections in five previously splenectomized children, that attention was focused on this risk. Still, increased susceptibility to infectious disease after splenectomy has not been generally acknowledged until quite recently (159).

### Overwhelming postsplenectomy infection

Since the report of King and Shumacker, presented almost thirty years ago (99), numerous patient series and case reports have confirmed the existence of a life-long threat to contract overwhelming postsplenectomy infections. This increased susceptibility to infectious disease after splenectomy shows a certain pattern

which, with the present knowledge, can be outlined roughly as follows: Characterized by sudden onset, rapid progression and resistance to therapy, these infections carry a high mortality rate (105). Singer (1973) in his review of 2 795 splenectomized patients concluded: "When septic disease strikes the asplenic individual, it is fulminant and the likelihood that death will result is considerable (58 %)" (159). A mortality rate of at least 50 % has been confirmed by several studies (15, 69, 176, 177). This is the most remarkable point as compared to the very low mortality of septic episodes in non-splenectomized individuals. In these latter, according to Singer (1973), the expected risk to die in septicemia is 0.07 % in children of 1-7 years of age, 0.02 % in the 5-14 year group and 0.01 % for the general population (159). These figures were estimated by extrapolating data for mortality due to meningitis and septicemia in childhood and for the general population in the U.S.A. According to the official statistics in Sweden the expected number of deaths in septicemia in children aged 1-14 is 6 per million, calculated as a mean during the period 1968-1976 (176).

The Waterhouse-Friderichsen syndrome (hemorrhagic adrenal necrosis), a manifestation of disseminated intravascular coagulation, is frequently present in fatal cases of postsplenectomy septicemia (24, 74, 105, 159). This is in contrast to patients with normal splenic function in whom disseminated intravascular coagulation secondary to pneumococcal infection is extremely rare (23). According to Whitaker (1969), the removal of splenic reticuloendothelial macrophages allows coagulation products, which otherwise should be phagocytized, to be present in the circulation and thereby enhance or induce intravascular coagulopathy (180).

Children, especially those with congenital and acquired anemia, portal hypertension, and lymphoreticular malignant tumors, are much more prone to develop postsplenectomy sepsis than are splenectomized adults. In e.g. Hodgkin's disease up to 10 % risk for children has been reported (34), whereas for older patients the risk is substantially less (50). However, some small series have shown the same risk whether the diseased individuals were splenectomized or not (55, 168). On the other hand, Askergrén and Björkholm (1980) reported pneumococcal septicemia in 6.6 % of adults subjected to staging laparotomy with splenectomy in Hodgkin's disease (12).

Traumatic rupture of the spleen is followed by less risk for fatal sepsis, than other reasons for splenectomy. However, this risk is at least 58 times that in the general population (159). Wählby and Domellöf (1981) found 10 incidents of septicemia when reviewing 413 children with traumatic rupture of the spleen (mean follow-up period 5.9 yr), and the mortality of the infection was 50 % (176). In a long-term follow-up of 740 American servicemen splenectomized because of trauma during the 1939-45 war, Robinette and Fraumeni (1977) showed a significant excess mortality (0.81 %) from pneumonia (144).

Fulminant sepsis is reported to occur predominantly shortly after splenectomy. In newborn infants and small children, the interval between splenectomy and subsequent infection seems to be shorter than for adolescents and adults (159). In children, between 30 and 80 % of all cases of fulminant septicemias have been reported to occur within two years after splenectomy (88, 145, 176, 177). Gopal and Bisno (1977) reported fulminant infections in five asplenic hosts without any severe underlying disease and reviewing the literature they found altogether 20 cases. The median interval between splenectomy and infection was three-and-a-half years and the mortality rate 75 % (74). Fatal sepsis is reported to occur up to 25 years after post-traumatic splenectomy performed in adults (77).

Taken together, published series including both pediatric and adult splenectomized groups, collected by Francke and Neu (1981) showed a morbidity of 3.9 % (4 846 patients) and a mortality of 2.4 % (5 485 patients) in postsplenectomy infections (69).

#### Organisms causing overwhelming postsplenectomy sepsis

Singer (1973) in his review of 2 795 splenectomized patients found Streptococcus pneumoniae in 48 % of the blood cultures taken during postsplenectomy sepsis. Other organisms, in decreasing order of frequency, were Neisseria meningitidis (12 %), Escherichia coli (11 %), Haemophilus influenzae (8 %), staphylococci (8 %) and streptococci (7 %) (159). This pneumococcal predominance has been confirmed by several authors (15, 74, 176).

#### Hematological changes after splenectomy

The hematological effects of splenectomy in humans have been studied most extensively concerning the red blood cells. Alterations of the red cell surface and presence of red cell inclusions, exemplified by target cells and Howell-Jolly bodies, respectively, provide evidence of asplenia (44). However, divergent results concerning these subtle morphologic findings possibly due to technical differences in the preparation of the specimens do not permit quantitative assessments of splenic activity (13, 137, 139).

Postsplenectomy leucocytosis, characterized by neutrophilia shortly after splenectomy, is followed by lymphocytosis and monocytosis during the following weeks (117, 139). Studies using surface marker tests for T and B lymphocytes in order to characterize the lymphocytosis after splenectomy in humans were not, to our knowledge, available at the time of the present investigation.

It is generally acknowledged that extirpation of the spleen is followed by a thrombocytosis. If normalization of the platelet count takes place later, or not, is, however, controversial (87, 139).



Splenectomized individuals have increased opsonic requirements for intravascular clearance of sensitized red blood cells and pneumococci (90) which after splenectomy takes place mainly in the liver (90). Being important opsonins the immunoglobulins have been quantified after splenectomy with contradictory results (32, 36, 100, 166, 185). However, deficiency of the tetrapeptide tuftsin (162) and, most likely, disturbance in immunoglobulin production (148), probably results in impaired opsonization and phagocytosis of antigenic particles. Clearance of pneumococci from the blood stream by the macrophages of the reticuloendothelial system also depends on complement for maximal efficiency (89). Both the alternative and classical pathways lead to deposition of activated complement factor C3, which is the major opsonin of the complement system (184). Animal experiments have shown that depletion of factors of both the classical and alternative pathways will greatly impair the clearance of bacteria from the blood stream (89). The role of the complement system in splenectomized humans as regards clearance of encapsulated bacteria from the blood is not fully understood.

#### Methods used to preserve splenic function

Although splenectomy because of surgical or nonsurgical splenic injury is followed by a lower risk of overwhelming infection compared with that after splenectomy for hemato-oncologic disorders, the consequences of such infections may be equally disastrous. Methods used to preserve splenic function are non-operative treatment, use of local hemostatic agents, splenorrhaphy, partial splenectomy, ligation of the splenic artery, and heterotopic autotransplantation of splenic tissue. If none of these methods are possible to perform, pneumococcal vaccination is advocated (60).

#### Nonoperative treatment

Splenic injuries after blunt abdominal trauma confirmed without laparotomy have been successfully treated nonoperatively in carefully selected patients. Diagnostic procedures include peritoneal lavage, utilization of radioisotope scanning and arteriography, the latter two increasingly used not only as diagnostics but also for follow-up studies of splenic injuries. Peritoneal lavage has a very high diagnostic accuracy (134) and has been used extensively in case of suspected intra-abdominal injury. However, this diagnostic tool seems to result in many unnecessary laparotomies which have favoured radionuclide scanning to become the basic laboratory diagnostic method (98). This is possible only if a portable gamma camera is available, allowing diagnostic radionuclide scans to be performed in the intensive care unit with no interruption in the continuity of resuscitation and monitoring (98). In special cases, ultrasound scanning of the abdomen or abdominal computerized tomography may be useful (165). Nearly 100 cases of nonoperative management have been reported in children with excellent results (56, 91, 98, 119, 165, 171). Thus, it seems established that nonopera-

tive treatment can be safe in the pediatric age group provided great care in patient selection is exercised and facilities for careful observations and follow-up are available. In accordance with other reports (124, 165) nonoperative treatment of splenic injuries in adults, verified by peritoneal lavage,  $^{99}\text{Tc}^m$  liver-spleen scan and/or arteriography, has been used at our surgical department since 1976 in 10 patients without any complications (unpublished). The criteria for nonintervention are stable vital signs, improving abdominal signs, and laboratory evidence of cessation of bleeding (124).

It must be emphasized, however, that one risk of nonoperative treatment is the possibility of missing a serious, associated injury. In addition, delayed rupture of the spleen, although rare and controversial, is another serious complication seen after splenic injury. Formerly believed to occur in about one fourth of the patients (57, 68, 107, 182), these entities probably represent more accurately a "delayed diagnosis", and a truer estimation of their incidence would be less than 5 % (17, 20, 132). The use of serial scans has made it possible to detect and to follow the course of a subcapsular hematoma after splenic injury (119). Bearing these facts in mind a successfully accomplished nonoperative treatment not only preserves splenic function but probably also reduces the risk of posttraumatic complications.

#### Operative treatment

"The salvaged spleen or splenic remnant in the left upper quadrant is a far greater trophy for the surgeon than the spleen in the pathologist's hands." (Leon Morgenstern 1981)

Blunt trauma usually produces a transverse tear with the plane vertical to the longitudinal axis of the spleen (78). However, injuries to the spleen range from minor evulsions of the capsule to simple transverse fractures, fractures into the hilus, longitudinal injuries, subcapsular hematomas, or total disruption of the parenchyma of the organ (157). This wide spectrum of damage to the spleen necessitates several different methods in attempt to preserve splenic function. Several topical hemostatic agents, including Gelfoam<sup>R</sup>, Surgicel<sup>R</sup>, oxidized cellulose (Oxycel<sup>R</sup>), and microfibrillar collagen, have been used for control of the bleeding splenic surface. The introduction of microfibrillar collagen, Avitene<sup>R</sup>, in 1973 together with growing awareness of the increased risk to contract serious infections after splenectomy, stimulated surgeons to avoid splenectomy whenever possible. Microfibrillar collagen has a more physiological basis for hemostatic activity, than other commonly used topical agents, forming an adherent lattice-like structure to which platelets are attracted and aggregate (122). Small capsular tears can effectively be treated with application of microfibrillar collagen provided that meticulous care is taken to use the agent in a proper way (79, 122, 156, 165, 179). Morgenstern (1977) reported successful use of topical hemo-

static agents in 19 of 21 patients with operative injury to the spleen (122). These results indicate that many incidental splenectomies can be avoided, thus reducing one of the major contributors to splenectomy. Weinstein et al (1979) reported 30 consecutive patients sustaining splenic injury from blunt abdominal trauma of whom 60 % had all or a portion of the injured spleen salvaged. The operative procedure was determined by the surgeon after evaluation of the damage to the spleen, consideration of the severity of associated injuries, and the general condition of the patient. Splenectomy was performed when the surgeon concluded that the spleen was not salvageable, or that in the process of saving it there would be an undue loss of blood or an inappropriate delay in the treatment of associated injuries (123, 179). Suture repair of splenic injury, although reported already at the end of the 19th century (147), was seldom used in adults until Morgenstern (1979) and Weinstein et al (1979) recommended the procedure (123, 179). Recently, Pachter et al (1981) reported successful splenorrhaphy in 24 of 27 consecutive patients (75 % adults) with splenic injury (133). Technically, there are many ways to carry out suture repair of the spleen. An extensive review was given by Buntain and Lynn (1979). The procedures include simple or mattress sutures with absorbable suture materials, possibly including omentum, or wrapping of the spleen either with omentum or a net of absorbable sutures (31). Rebleeding after splenorrhaphy necessitating splenectomy has been reported twice (98, 172). Experimental studies in rats have shown that after a standardized splenic trauma primary splenic repair is a safe method and renders normal resistance to pneumococcal challenge (78). It must be emphasized, however, that no study comparing suture repair and ligation of bleeding vessels combined with microfibrillar collagen has been performed. Perhaps suture repair is not only unnecessary, but also produces devitalized splenic tissue.

Extensive studies in humans and animals have revealed the segmental end vessel distribution of splenic arteries (see 54). Gupta et al (1976) made casts of human splenic arteries by injection of butyl butyrate and found after removal of the parenchyma that more than 80 % of the specimens demonstrated two primary lobar intrasplenic branches. In the rest there were three primary lobar branches (80). These observations form the basis for segmental resection of the spleen. Though performed before, Christo (1962) pioneered partial resection of the spleen for trauma and reported eight cases (35). In the 1970:s there have been numerous reports on successful splenic resections performed not only in children but also in adults (79, 123, 133, 156, 165, 179). Experimental studies performed in rats have shown that the remnant after splenic resection will offer protection against pneumococcal infection (41, 71, 76, 78). The degree of protection is dependent on the amount of splenic tissue left and the postoperative interval before challenge (7, 71, 174). Thus, experience in both animals and humans shows that splenorrhaphy or partial splenectomy is safe and probably protects against overwhelming postsplenectomy infection. This fact, together with



the awareness of the increased risk to contract septicemia after staging procedures with splenectomy in Hodgkin's disease, has initiated attempts to preserve splenic function in these patients (25). However, when reviewing 320 consecutive splenectomies in staging laparotomies for Hodgkin's disease, Dearth et al (1978) found splenic involvement to be limited to a few nodules in a localized distribution without visible subcapsular lesions in 11.6 % of spleens with disease (49). Therefore, splenic involvement by Hodgkin's disease cannot be confidently excluded by performance of partial splenectomy. The risk of understaging must critically be evaluated together with the risk to contract serious postsplenectomy infections after total splenectomy. In the future, perhaps computer tomography safely can exclude splenic involvement, thus making splenectomy unnecessary.

In cases of massively bleeding splenic trauma, involving major segmental vessels, successful treatment in both animals and children by splenic artery ligation has been reported in combination with suturing of the lacerations (40, 95, 96). Spleen scans after three weeks or more were normal. Experiments performed in rats show that splenic weight eight weeks after splenic artery ligation is 47 % of that of spleens from control animals. Antibody titers were considerably higher than those of splenectomized rats but lower than sham operated animals injected intravenously with sheep red blood cells (173). Immunization with formalin-killed pneumococci three months after splenic artery ligation resulted in prolonged survival time after pneumococcal challenge as compared with totally splenectomized rats or splenectomized rats with splenic implants immunized in an identical way (42).

When splenectomy is considered necessary, heterotopic autotransplantation of splenic tissue has been suggested as a method to preserve splenic function. Splenosis, i.e. multiple small splenic nodules throughout the peritoneal cavity, was first described in man by Albrecht in 1896 (4). In the beginning of the 20th century several case reports of splenosis following abdominal injury were published (see 29). Performing experiments in rabbits, Marine and Manley, already in 1917 (with further data in 1920) demonstrated the growth of subcutaneously placed splenic autografts (113). Jacob et al (1963) showed that the growth and function of spleen autotransplants in rats, as measured by their size and by their capacity to sequester <sup>51</sup>Cr-labelled altered red cells, were regulated in proportion to the amount of particulate matter presented, i.e. "work load" (92).

Hence, much work on preservation of splenic functions has been reported. Likewise, the effect of previous immunization on susceptibility to pneumococci has been extensively studied in animals (21, 41, 42, 66, 84, 106). However, efforts to improve treatment of overwhelming postsplenectomy infection using an animal model have not yet been presented.

## Animal models for the study of overwhelming postsplenectomy infection

Several animal models have been developed in order to study the pathogenesis of overwhelming postsplenectomy infection (Table 1). Morris and Bullock (1919) showed that both young and old splenectomized rats were much more prone to contract fatal sepsis due to the bacillus of rat plague (*Pasteurella* sp.?) (125). Causing nine tenths of the mortality of rats under normal laboratory conditions, this infection was well recognized. Compared to control animals, splenectomized rats exhibited a significantly increased spontaneous mortality due to infections. After subcutaneous injection of the bacillus a seven fold increase in mortality was demonstrated after splenectomy. Since then most animal studies have been carried out with *Streptococcus pneumoniae* as the infective organism (Table 1). Shinefield et al (1966) and Whitaker (1968) reported increased susceptibility of splenectomized mice to pneumococci injected intravenously (158, 181). Shinefield et al (1966) showed that after pneumococcal challenge bacterial multiplication subsequently occurred in a higher proportion in splenectomized mice and at a more rapid rate than in control animals (158). In the rat, clearance of  $0.5 \times 10^6$  colony forming units (CFU) of *Streptococcus pneumoniae* type 1 from the blood stream after intravenous challenge takes place within eight hours in control animals. In contrast splenectomized rats show a progressive increase in number of viable pneumococci per milliliter of blood during the same time (43). A much less impressive increase in susceptibility was noted after intraperitoneal administration of the bacteria. Also, increased susceptibility of splenectomized mice to infection after exposure to an aerosolized suspension of pneumococci has been demonstrated (38). Reports on the regeneration of heterotopic autotransplanted splenic tissue (92, 113, 167) and its protection against *Bartonella muris* infection (46, 140) stimulated studies on protective effect against pneumococcal infection. However, these studies have produced contradictory results (43, 75, 110, 154).

Table 1. Animal models used for the study of overwhelming postsplenectomy infection.

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| <u>Research group</u>          | <u>Bacteria</u>                                  | <u>Animals</u> |
|--------------------------------|--------------------------------------------------|----------------|
| Morris and Bullock, 1919 (125) | <i>Bacillus of rat plague (Pasteurella sp.?)</i> | rats           |
| Shinefield et al., 1966 (158)  | <i>Streptococcus pneumoniae</i> type 6           | mice           |
| Schulkind et al., 1967 (151)   | <i>Streptococcus pneumoniae</i> type 1           | rabbits        |
| Whitaker, 1968 (181)           | <i>Streptococcus pneumoniae</i> untyped          | mice           |
| Haller, 1970 (84)              | <i>Streptococcus pneumoniae</i> not specified    | rats           |
| Biggar et al., 1972 (21)       | <i>Streptococcus pneumoniae</i> type 3           | rats           |
| Leung et al., 1972 (106)       | <i>Streptococcus pneumoniae</i> type 25          | rats           |
| Coil et al., 1978 (38)         | <i>Streptococcus pneumoniae</i> type 3           | mice           |
| Likhite, 1978 (110)            | <i>Streptococcus pneumoniae</i> type 3           | mice           |
| Schwartz et al., 1978 (154)    | <i>Streptococcus pneumoniae</i> type 25          | rats           |
| Cooney et al., 1979 (41-43)    | <i>Streptococcus pneumoniae</i> type 1           | rats           |
| Van Wyck et al., 1980 (174)    | <i>Streptococcus pneumoniae</i> type 3           | rats           |
| Greco and Alvarez, 1981 (76)   | <i>Streptococcus pneumoniae</i> type 25          | rats           |

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## AIM OF THE PRESENT STUDY

Hematological and immunological findings after splenectomy in humans are a matter of controversy. This probably can be explained by the heterogeneity of the patient materials, most often including individuals with hematological and other underlying diseases. Hence, it was one of the aims of the present study to investigate hematological alterations and immunocompetence after splenectomy in patients without the influence of concomitant diseases (I).

An animal model was developed to elucidate the role of the spleen in the defence against intravenously injected pneumococci. The purpose was to get a reliable model resembling the clinical condition of overwhelming postsplenectomy infection (II).

This animal model was used to investigate:

- the influence of postsplenectomy interval on susceptibility to bacterial challenge (III);
- splenic resection as alternative to splenectomy and surgical procedures for heterotopic transplantation of splenic tissue (IV);
- the effect of steroids on survival in overwhelming postsplenectomy infection (V).

## MATERIAL AND METHODS

### Patients (I)

At the Department of Surgery, University of Lund, 377 patients were operated with highly selective vagotomy because of chronic duodenal ulceration during 1971 to 1978. In 13 (3.4 %) of these patients splenectomy was performed because of inadvertent splenic injury during the operation. One splenectomized patient died during the follow-up period in prostatic carcinoma. A control patient to each of the 12 remaining splenectomized patients, operated the same year and of the same sex and similar age was chosen (see paper I, Table 1).

### Laboratory investigations

Hemoglobin concentration, total differential leucocyte counts and platelet counts were determined by standard techniques as used at our hospital.

Plasma concentration of IgG, IgA, IgM and complement factors C3 and C4 were determined by electroimmuno assays (104).

A hemolysis-in-gel assay was used for screening defects in the classical and alternative pathways of complement activation (170).

Tests for lymphocyte subsets were carried out on heparinized blood:

- a) For detection of immunoglobulin-bearing cells, the diluted blood sample was first centrifuged at 400 g, 30 min on Isopaque-Ficoll (Lymphoprep<sup>R</sup>, Nyegaard, Oslo). The cells at the interphase were incubated with latex particles at 37°C, washed and made up to  $4 \times 10^6$  cells/ml. FITC-labelled F(ab')<sub>2</sub>-fragments of goat antibodies to human immunoglobulins (reagents obtained from Kallestad Laboratories Inc., Kemila, Stockholm, Sweden) were used in a direct immunofluorescence assay. In one test, an anti-human immunoglobulin (heavy and light chains) reagent was used and in another a reagent specific for IgM (μchains). Two hundred cells were counted in a Leitz Ortholux microscope with combined phase-contrast and UV light illumination. Cells with engulfed latex particles were not counted.
- b) For rosetting experiments, the blood was mixed with methyl cellulose and carbonyl iron. After sedimentation at 37°C, collection of the upper phase was followed by centrifugation on Lymphoprep<sup>R</sup> as described above. The cells at the interphase were washed and made up to  $2 \times 10^6$ /ml.

T-lymphocytes were demonstrated in a rosetting test with untreated sheep red blood cells obtained from a single

animal (82).

Lymphocyte receptors for Fc of IgG(Fcγ) were demonstrated in a rosetting test using bovine erythrocytes sensitized with rabbit antibodies as indicator cells (82, 83).

Lymphocyte receptors for complement were demonstrated in a rosetting test using bovine erythrocytes sensitized with rabbit antbovine erythrocyte antibodies of the IgM class and rabbit complement from a C6-deficient rabbit (83).

T-cells with receptors for Fc of IgG were demonstrated in a rosetting test where a mixture of fluorescein-labelled sheep erythrocytes and unlabelled bovine erythrocytes coated with IgG was centrifuged with lymphocytes. The rosetted lymphocytes were scored as fluorescent, non-fluorescent, or mixed, using a UV/phase contrast microscope (82).

## Animal models

### Animals

Male Sprague-Dawley rats were used. During the experiments they had free access to water and standard food pellets.

The animals were anesthetized with ether during operations and injections.

### Splenectomy (II-V)

The operation was performed via an upper midline incision, the spleen was mobilized into the wound and the vessels ligated close to the organ using three to four 4-0 silk sutures.

### Sham operation (II-V)

The surgical procedure included mobilization of the spleen as described above, but the spleen was put back to its normal position avoiding damage to the parenchyma or the vessels.

### Omental resection (III)

The greater omentum was mobilized through a midline incision and about two-thirds resected after individual ligation of three to four vessels using 4-0 silk sutures.

### Splenic resection and heterotopic autotransplantation of splenic tissue (IV)

These operations were also performed via an upper midline incision.



Resection of the upper two-thirds of the spleen was performed after individual ligation of the vessels to this part. Bleeding stopped spontaneously from the remnant.

The spleen tissue used for heterotopic autotransplantation of splenic tissue was prepared in two different ways: either by dividing the whole spleen into 12 equal pieces, or by crushing and dispersing it in 1 ml of sterile saline. The tissue pieces were implanted (a) subcutaneously, six on each side of the midline incision, into pouches prepared using a pair of scissors or (b) sutured to the greater omentum with plain 4-0 catgut. The dispersed splenic tissue was injected (a) into both rectus muscles, (b) subcutaneously on both sides of the incision used for splenectomy, or (c) extra-peritoneally in the left flank just below the diaphragm.

#### Heterotopic homologous transplantation of splenic tissue (III).

Dispersed splenic tissue, taken from animals of the same age and similar weight, was implanted intraabdominally into previously splenectomized animals immediately before bacterial challenge (see below).

#### Preparation and storage of pneumococci

Streptococcus pneumoniae type 1 (obtained from Statens Seruminstitut, Copenhagen, Dr J. Henrichsen) was cultured in Todd Hewitt broth for 15 hours. The pellet was suspended in serum broth (1 part of fetal calf serum + 5 parts of Todd Hewitt broth) to the extinction 0.70 in a Linson 3 photometer (fresh serum broth as blank). After incubation for 90 minutes in a 37°C water bath, dilutions of  $10^4$ ,  $10^5$  and  $10^6$  were prepared keeping the tubes on ice. The bacterial suspensions were divided into 1 ml aliquots, quickly frozen using liquid nitrogen and stored at -80°C. The number of colony forming units (CFU) of Streptococcus pneumoniae was then determined for each dilution.

Before being used for injection, the suspensions were thawed in the hand, diluted in sterile saline and then kept on ice. The viable count was checked regularly.

#### Challenge of the animals with pneumococci (II-V)

Intravenous injections of pneumococci were performed in the lateral vein of the hind limb. This procedure succeeded in most animals, but in about 10 % the penile vein had to be used. A quarter of a milliliter (II and V) or half a milliliter (III and IV) of appropriately diluted bacteria was used.

#### Blood cultures

Autopsy was performed in all animals which died during the experiments. The thoracic cavity was opened first, the heart apex was

then cut off and the heart blood was inoculated on hematin agar plates. Growing bacteria were identified after 24 hours' incubation at 37°C.

#### Histological examination of the splenic tissue in experiments with resection and heterotopic autotransplantation (IV)

The splenic residuals were cut out and fixed in 10 % formalin. Thin slices were then embedded in paraffin blocks from which tissue sections of 4  $\mu$ m were made. These sections were stained with hematoxylin and eosin and by the modified method of van Gieson for collagen fibres and Gomori's iron reaction. The tissue sections were studied in a light microscope.

#### Drugs used for treatment of postsplenectomy pneumococcal sepsis (V)

The administration procedures as well as substances and doses used are described in paper V.

#### Statistical analysis

In the clinical study the data obtained were analyzed using the student's t-test for unpaired observations.

In the experimental study the Mann-Whitney rank-sum test for unpaired data was used to evaluate survival times and Fisher's exact (probability) test to compare mortality rates.

## RESULTS AND DISCUSSION

### Immunocompetence after incidental splenectomy in patients undergoing highly selective vagotomy (I)

Theoretically, immunological and/or hematological diseases may be associated with chronic duodenal ulceration. It is, however, unlikely that this should influence on our evaluation of the immunocompetence after splenectomy.

The addition of splenectomy to a surgical procedure will greatly contribute to the morbidity and mortality attending the primary operation (37, 48, 51, 65, 186). As might have been expected, the 12 patients subjected to incidental splenectomy during operation for chronic duodenal ulceration showed a significantly longer postoperative hospital stay than that of matched controls. In evaluating 97 patients undergoing a Nissen fundoplication because of esophageal reflux Rogers et al (1980) found a significantly higher morbidity rate in those 25 patients who had an incidental splenectomy (146). Whether this increased morbidity and mortality, seen after incidental splenectomy, is due to absence of normal (or diminished) splenic function or to the surgical trauma per se is not fully clarified. It is reasonable to claim that the increased incidence of thromboembolic complications after splenectomy appears as a result of a postsplenectomy thrombocytosis since platelet counts exceeding 400 000 per cubic millimeter increase the risk tenfold (169).

Table 2 summarizes the hematological findings in incidentally splenectomized patients as compared to control subjects. The hemoglobin concentration did not differ between the two groups. However, the number of lymphocytes, monocytes and platelets were increased in splenectomized individuals. Thus the number of lymphocytes was about 50 % higher and the number of platelets almost twice as high when compared to the controls.

Table 2. Hematological findings in splenectomized patients as compared to control subjects (summarized from paper I).

| Decreased | Unchanged                | Increased            |
|-----------|--------------------------|----------------------|
| IgM       | Hemoglobin concentration | White blood cells    |
|           | Neutrophils              | Lymphocytes          |
|           | Eosinophils              | Monocytes            |
|           | Basophils                | Platelets            |
|           | IgA                      | IgG                  |
|           | Complement factor C4     | Complement factor C3 |

Serum screen test for classical and alternative complement pathways were normal in all patients.



Increased total leucocyte counts have been demonstrated three months or more after splenectomy in patients free from underlying hematological disorders (117). In our series, the leucocytosis still persisted almost five years after the operation (I). The study confirmed other reports (44, 117) indicating that the rise in total leucocyte counts is due to increased numbers of lymphocytes and monocytes rather than of granulocytes.

Splenectomy is usually followed by a mild thrombocytosis which reaches a peak between the fourth to thirteenth postoperative day (44). Hirsh and Dacie (1966) suggested that persistent thrombocytosis after splenectomy is a consequence of continuing anemia in association with active hemopoiesis (87). However, after incidental splenectomy our patients showed platelet counts that were significantly increased ( $401 \pm 46$  compared to  $223 \pm 90 \times 10^9/l$  for control patients) almost five years after splenectomy, without any corresponding anemia (I).

Compared with the control patients, 20 % increase in IgG and a 36 % decrease in IgM were found in splenectomized individuals, while the IgA levels were unchanged. Immunoglobulin levels after splenectomy have been studied with apparently contradictory results (32, 36, 100, 166, 185). According to Constantoulakis et al (1978) splenectomy produces a decrease in IgM which persists after one year and returns to normal four years later (39). In our patients subjected to highly selective vagotomy because of chronic duodenal ulceration a decrease in IgM and increase in IgG were found after five years in those incidentally splenectomized (I).

Anatomically or functionally asplenic patients have a deficient primary antibody response to intravenously injected antigenic particles. Rowley (1950), when injecting heterologous red blood cells (148) and Sullivan et al (1978) injecting bacteriophage (166) intravenously found impaired primary antibody production in splenectomized individuals. In contrast, subcutaneously administered antigens have been reported to produce normal antibody responses both in man and animal previously splenectomized (8, 21, 166). Lozzio and Wargon (1974) reported that congenitally asplenic mice have a marked deficiency of immunoglobulin production not only in primary but also in secondary immune response to sheep erythrocytes (111). Also in humans, a deficient secondary immune response has been noted, producing IgM rather than IgG antibodies after secondary intravenous immunization (166).

In splenectomized patients and animals, there is an increased opsonic requirement for clearance of heterologous erythrocytes and pneumococci and though serum from splenectomized patients shows normal ability to opsonize pneumococci in vitro (183), this is probably not sufficient to compensate for the reduced phagocytic efficiency after splenectomy (90). The lack of splenic phagocytes can be compensated for by the liver if increased amounts of specific antibodies are present (90). However, deficiency in antibody

production is not sufficient in itself to account for the septicemia seen in splenectomized persons (90). Decreased levels of the tetrapeptide tuftsin, which is seen not only after splenectomy but also in functional asplenia, probably contribute to the increased susceptibility to infectious disease (129, 130). Low levels of tuftsin are noted after elective removal of the spleen for therapeutic or staging purposes, whereas splenectomy after accidental rupture of the spleen does not usually result in tuftsin deficiency (129, 162). However, further studies are required to evaluate the precise role of tuftsin and immunoglobulins in overwhelming infection after splenectomy.

Apart from constituting the removal of a major organ for IgM synthesis (149), the question also arises whether splenectomy may lead to a derangement of the normal cooperation between subsets of immunocompetent cells responsible for the immunoglobulin production. This possibility has been investigated in mice, but it is still a matter of controversy if removal of the spleen leads to an aberration in cooperation between T and B cells (9, 149, 178).

In our study the number of both T and B lymphocytes was raised in patients five years after incidental splenectomy compared to non-splenectomized control subjects. In 37 splenectomized patients, Millard and Banerjee (1979) found 54 % to have raised T and/or B cells. Follow-up data indicated that the raised levels of T and/or B blood lymphocytes were seen mainly in patients splenectomized several years earlier (118).

Hence, sufficient numbers of immunocompetent cells seem to be present after splenectomy, at least in the peripheral blood. Further analysis of lymphocyte subsets in the peripheral blood demonstrated a significant increase in lymphocytes with receptors for Fc of IgG (Fc $\gamma$ ) in splenectomized patients as compared to controls (I). Since cells with Fc $\gamma$  receptors comprise a heterogeneous population this finding is hard to evaluate. Even more so since the Fc $\gamma$  receptor may be a differentiation marker, the expression of which may vary within a functional population. Likewise lymphocytes with receptors for complement represent a heterogeneous population. The number of these cells did not, however, differ between splenectomized and control subjects. Using a "mixed rosette" procedure with IgG-coated bovine erythrocytes and sheep erythrocytes (fluorescein labelled) it was demonstrated that also within the T cell population the proportion of cells with Fc $\gamma$  receptors was increased (I). This subset of T cells was originally demonstrated to contain T suppressor cells (121). However, later studies have questioned the Fc $\gamma$  receptor as a functional marker among T cells (see 59). It has been demonstrated that antibody deficiency may be the result of excess suppression (see 59). The present data do not answer if this is so also after splenectomy, but further functional analysis of this issue seems warranted. The recent development of monoclonal antibodies to functionally

restricted lymphocyte subsets (59) may further clarify the significance of increased numbers of T cells with Fc $\gamma$  receptors in splenectomized individuals.

Screening by the hemolysis-in-gel technique showed no serious defects in the classical and alternative complement pathways in the splenectomized individuals (I). Clearly aberrant patterns are observed in approximately 4 % of healthy blood donors with this technique (170). Quantitation of complement factor C3 revealed an increase (mean 23 %) in splenectomized patients, whereas the level of complement factor C4 did not differ from that of the control individuals (I). However, at least in non-splenectomized animals, marked deficiency of complement is required before clearance of pneumococci is impaired. Even in patients with congenital C3 deficiency, who suffer from bacterial infections, the syndrome of overwhelming sepsis is not a characteristic feature (6, 16, 90). Hence, the present study did not support the possibility that a defect of the complement system is responsible for the increased incidence of infections after splenectomy.

#### Effect of intravenous injection of *Streptococcus pneumoniae* type 1 in splenectomized rats (II, III)

The inability of splenectomized individuals to handle hematogenously spread bacteria has not yet been satisfactorily clarified. Hence, a splenectomized-rat model was developed to elucidate the overwhelming postsplenectomy sepsis problem. *Streptococcus pneumoniae* being the most prevalent encapsulated bacteria involved in these infections, was used to produce an experimental sepsis. To facilitate these studies attempts were made to standardize the bacterial inocula in order to retain the viability and virulence of the pneumococci during storage.

Animals splenectomized 18 weeks before pneumococcal challenge were extremely susceptible to these bacteria injected intravenously. As little as 200 CFU of *Streptococcus pneumoniae* type 1 produced overwhelming postsplenectomy infection with a high mortality rate (80 - 100 %). When  $2 \times 10^3$  and  $2 \times 10^4$  CFU were given, all animals died and their survival times were inversely correlated to the dosis given (Table 3). In contrast, eight splenectomized rats injected with sterile serum broth all survived, as did all of the 41 sham operated rats, irrespectively of the pneumococcal dosis given (between  $2 \times 10^2$  and  $2 \times 10^4$  CFU).

The majority of animal studies concerning postsplenectomy infection have been performed in rats (Table 1). The methods generally in use when studying pneumococcal challenge in the splenectomized rat model are based on preparation of fresh bacterial inocula for each experiment. Thus, overnight incubation of bacteria, reinoculation and harvesting during log phase growth, washing and dilution to an appropriate concentration of bacteria are required before each ex-

Table 3. Effect of intravenous injection of various inocula of *Streptococcus pneumoniae*, type 1, stored at  $-80^{\circ}\text{C}$ , on the mortality and survival time of splenectomized rats (from paper II).<sup>1</sup>

| <u>Number of CFU inoculated</u>             | <u>Number of rats killed/injected</u> | <u>Median survival time (range) of killed animals, h</u> |
|---------------------------------------------|---------------------------------------|----------------------------------------------------------|
| $2 \times 10^4$                             | 8/8                                   | 27 (22-96)                                               |
| $2 \times 10^3$                             | 8/8                                   | 31 (29-90)                                               |
| $2 \times 10^2$                             | 8/8                                   | 36 (34-132)                                              |
| $2 \times 10^2$ (after storage of 6 months) | 8/10                                  | 33 (26-48)                                               |
| Serum broth                                 | 0/8                                   | > 4 weeks                                                |

<sup>1</sup> Sham-operated rats injected with  $2 \times 10^4$ ,  $2 \times 10^3$ , and  $2 \times 10^2$  CFU pneumococci or serum broth (8 animals in each group), all survived until sacrificed after 4 weeks.

Table 4. Lack of effect of postsplenectomy interval on mortality and survival time in Sprague-Dawley rats challenged with  $4 \times 10^3$  CFU of *Streptococcus pneumoniae* type 1 (from paper III).

| <u>Postsplenectomy interval (weeks)</u> | <u>n</u> | <u>Number of killed animals</u> | <u>Median (range) survival time in killed animals (h)</u> |
|-----------------------------------------|----------|---------------------------------|-----------------------------------------------------------|
| 8                                       | 15       | 15                              | 25.5 (25.5 - 40.2)                                        |
| 1                                       | 15       | 15                              | 26.0 (23.0 - 42.3)                                        |



periment. Hence, the exact amount of bacteria in the preparation is unknown at the time of challenge of the animals. In addition, the bacteria are repeatedly passaged in rats or mice in order to preserve virulence and encapsulation since subcultivation on solid agar often results in changes in these characteristics (21, 41, 106). The use of frozen bacterial inocula, recently introduced in the field of group A streptococci (163), made it possible to select a predictable inoculum at each occasion, thus minimizing the number of animals needed for investigation of the overwhelming post-splenectomy pneumococcal infection problem (II-V). Furthermore, it was possible to retain the pneumococcal virulence for the animals. After six months storage, intravenous injection of  $2 \times 10^2$  CFU of pneumococci still killed eight out of ten splenectomized animals (Table 3).

This, and other studies thus amply demonstrated the importance of the spleen in resistance to intravenously injected, virulent pneumococci (II, 21, 73, 106, 151).

In studies demonstrating an increased susceptibility of splenectomized rats to intravenously injected pneumococci, the time interval between splenectomy and pneumococcal challenge of the animals has ranged from five days (174) to six months (72). However, to our knowledge, the influence of the postsplenectomy interval on susceptibility to pneumococci has not been studied in the rat before.

Shinefield et al (1966) injected S. pneumoniae type 6 intravenously into pathogen free mice splenectomized five months or four days earlier. After repeating the experiments comparing a postsplenectomy interval of four hours and four days, without finding any differences, it was concluded that the increased susceptibility to infection with S. pneumoniae was not confined to young (4-week-old) mice with splenectomy and the deleterious effects of splenectomy did not disappear with time (158).

Intravenous injection of  $4 \times 10^3$  CFU of S. pneumoniae type 1 into rats splenectomized one or eight weeks earlier (15 animals in each group) killed all animals in both groups (Table 4). The survival time did not show any variation with the postsplenectomy interval (III).

Experiments were also performed in which pneumococci were injected peroperatively, either immediately after splenectomy or in animals splenectomized two weeks previously and subjected to omental resection at the time of bacterial challenge. A significantly higher survival rate was noted as compared with splenectomized animals not undergoing an operation at the time of challenge. The survival time in the succumbing animals challenged peroperatively was not influenced by postsplenectomy interval or the intraabdominal deposition of splenic tissue (Tables 5 and 6).

Table 5. Comparison of immediate and delayed postsplenectomy challenge with  $4 \times 10^3$  CFU of *Streptococcus pneumoniae* type 1. Mortality and survival time in Sprague-Dawley rats (from paper III).

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| <u>Postsplenectomy interval (weeks)</u> | <u>n</u> | <u>Number of killed animals</u> | <u>Median (range) survival time in killed animals (h)</u> |               |
|-----------------------------------------|----------|---------------------------------|-----------------------------------------------------------|---------------|
| 2                                       | 31       | 23                              | 28.5                                                      | (23.0 - 62.0) |
| 0                                       | 31       | 23                              | 30.0                                                      | (22.5 - 72.0) |

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Table 6. Effect of intraabdominal deposition of splenic tissue on mortality and survival time after challenge with  $4 \times 10^3$  CFU of *Streptococcus pneumoniae* type 1 in Sprague-Dawley rats splenectomized two weeks earlier (from paper III).

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|                                    | <u>n</u> | <u>Number of killed animals</u> | <u>Median (range) survival time in killed animals (h)</u> |               |
|------------------------------------|----------|---------------------------------|-----------------------------------------------------------|---------------|
| Intraabdominal deposition          | 17       | 16                              | 30.0                                                      | (25.0 - 72.0) |
| Sham operation (omental resection) | 20       | 15                              | 28.5                                                      | (25.0 - 53.5) |

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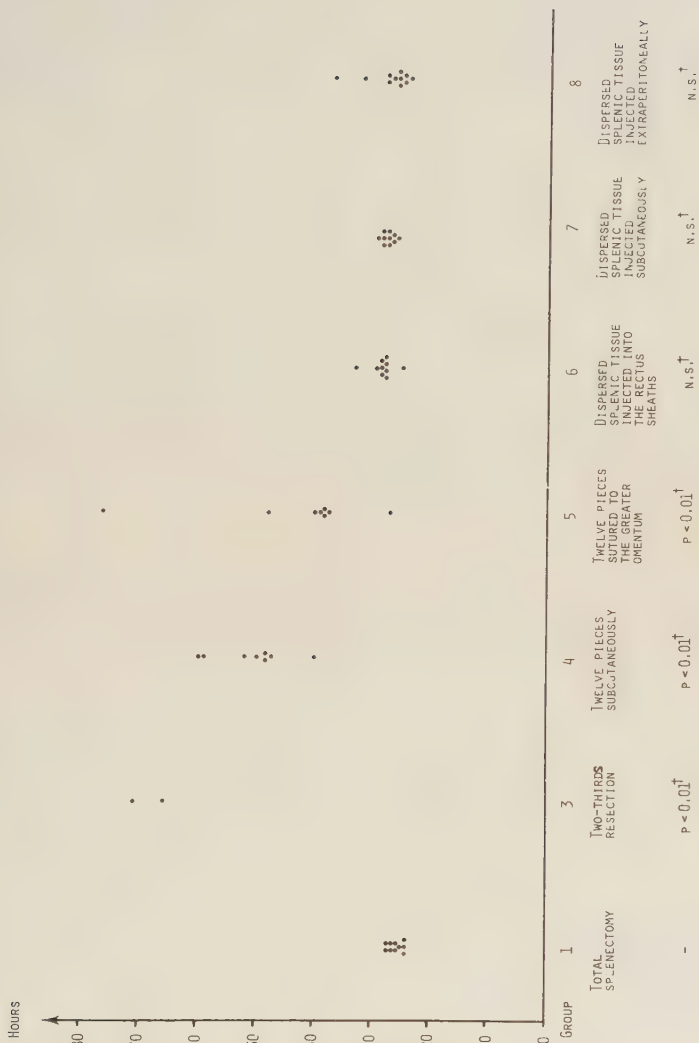
Similar experiments in mice implanted intraperitoneally with splenic tissue or 0.5 ml of pooled serum from normal mice, did not restore resistance of animals with splenectomy to intravenously injected pneumococci (158). Taken together our findings do not support the presence of splenic humoral factors contributing to resistance to infection with *S. pneumoniae*, unless this (unknown) factor would have an extremely short life-time in the circulation. It seems, however, as if a surgical procedure *per se* has a positive influence on survival rate after pneumococcal challenge (III). This may possibly be explained by an increase in C-reactive protein (CRP) known to occur after surgical trauma (10). CRP has binding specificity for the phosphocholine determinant of the cell wall C-polysaccharide from *S. pneumoniae* (1, 175). This can lead to complement activation in human serum by the classical pathway (94). In a recent study on mice Mold et al (1981) provide direct evidence of the effectiveness of CRP in host defence against pneumococcal infection (120). Furthermore, an association has been reported between elevated levels of CRP and increased resistance to *Staphylococcus aureus* infection in endotoxin-treated mice (136).

#### Splenic resection and heterotopic autotransplantation of splenic tissue: regeneration and protection against pneumococcal challenge (IV)

The ability of the spleen to regenerate in a heterotopic location without preserved arterial and venous circulation has been known for a long time. In animals, heterotopic autotransplanted splenic tissue has been shown to produce antibodies following intravenously injected sheep red blood cells and pneumococci (26, 41, 108, 153). Consequently, when splenectomy is inevitable, heterotopic deposition of the spleen, or a part of it, has been suggested as a method for preservation of splenic functions. However, different surgical procedures for splenic autotransplantation should be critically evaluated in relation to other methods of splenic conservation, e.g. partial resection, as regards regeneration and protective effect during bacteremia.

Sixteen weeks after two-thirds resection of the spleen a significant increase in weight of the splenic remnant was noted (44 %). Intravenous injection of  $4 \times 10^3$  CFU of *S. pneumoniae* type 1 at that time resulted in two deaths among 10 animals as compared with no surviving of 10 totally splenectomized rats. In addition, the succumbed spleen resected animals showed survival times of 65 h and 70 h, respectively, and those totally splenectomized 25 h (median, range 24 to 29) (Fig 1).

FIG. 1 SURVIVAL TIMES OF KILLED ANIMALS AFTER INTRAVENOUS INJECTION OF  $4 \times 10^3$  CFU OF  
*STREPTOCOCCUS PNEUMONIAE* TYPE 1, 16 WEEKS AFTER THE SURGICAL PROCEDURES (FROM  
 PAPER IV).



<sup>†</sup> = COMPARED WITH TOTAL SPLENECTOMY



Van Wyck et al (1978) investigated regeneration and antibody production against intravenously injected sheep red blood cells in rats after various degrees of partial splenectomy. Nine days after the surgical procedures, weight gain of splenic remnants was 139 % to 412 %, with the greatest percentage rise in the smallest remnants, compared with 58 % increase in spleen weight of sham-operated animals (173). The discrepancy between these and our results probably reflects the difference in age (weight) between the animals. Thus, our rats were about twice the weight as those in the experiments of van Wyck et al (180 to 250 g compared with 75-150 g). Furthermore, in our experiments sham operated rats showed no increase in splenic weight during the 16 postoperative weeks, suggesting that they were fully developed at the time of the surgical procedure. Van Wyck et al (1978) also demonstrated a positive relationship between splenic weight and log antibody titer. If less than one third of the spleen was left, the antibody titers were similar to the levels in rats without spleen (173). Six months after 75 % splenic resection Goldthorn et al (1978) evaluating resistance to pneumococcal challenge found these rats to have a susceptibility (mortality) roughly intermediate between that of total splenectomized and sham operated animals (72). When pneumococcal injections were performed after two weeks no difference in susceptibility could be registered, but after three weeks there was a significantly increased survival after 75 % splenic resection as compared with total splenectomy (71).

When the spleen was divided into 12 equal pieces and placed either subcutaneously or sutured to the greater omentum, regeneration was seen. The integrated weights of implanted pieces recorded at autopsy 16 weeks later were about one-third of that of the normal spleen (IV). In addition, microscopical examination showed in all cases a preserved architecture of the splenic implants.

Studies in the rat by Tavasolli et al (1973) have shown that the growth of splenic tissue in an ectopic location consists of a degenerative phase followed by regeneration. The regeneration starts in the outer zone of the implant and proceeds towards the center where necrosis is present. Central necrosis will proceed in large implants producing an environment not suitable for splenic regenerative processes, suggesting that the regeneration is optimal when very small splenic pieces are used (167). This was, however, not confirmed when dispersed splenic tissue was injected subcutaneously, intramuscularly or retroperitoneally in our study; only some small noduli (less than one mm in diameter) could be identified (IV).

It has been a matter of controversy whether heterotopically transplanted splenic tissue will protect also against overwhelming pneumococcal infection (43, 75, 110, 154). These divergent opinions probably more reflect experimental difficulties than true differences. The ability to standardize the bacterial inocula made it possible for us to study the protective effect of splenic implants

in the rat. Sixteen weeks after implanting the spleen, divided into 12 equal pieces, subcutaneously or into the greater omentum,  $4 \times 10^3$  CFU of pneumococci were injected intravenously. Prolonged survival time of rats with splenic implants was seen as compared with totally splenectomized rats after pneumococcal challenge (Fig 1). This prolonged survival time is evident only when small amounts of pneumococci are used for challenge (41), which may explain the different results reported in the literature concerning protective effect of autotransplanted splenic tissue.

As might have been expected from the negligible regeneration, animals with dispersed splenic tissue showed no increase in survival time after pneumococcal challenge as compared with totally splenectomized rats (Fig 1).

However, neither implantation of splenic pieces nor dispersed splenic tissue saved the animals from overwhelming pneumococcal sepsis and the mortality rate was the same as for animals without splenic tissue.

Thus, the resistance to pneumococcal challenge offered by heterotopic autotransplanted splenic tissue was only reflected in a prolonged survival time. This protection, though superior to the asplenic state would seem to be inferior to that recovered after splenic artery ligation, partial splenic resection or sham operation (41, 42). Recently, Fashing and Cooney (1980) reported a long term study in rats subjected to splenic heterotopic autotransplantation and immunization against pneumococci followed by re-immunization one year later. Improved survival after pneumococcal challenge in rats with splenic implants compared to those without splenic tissue suggests that autotransplantation and antipneumococcal vaccine should be combined (66).

Splenosis in man has been reported in about 80 cases in the world's literature up to 1973, but it is doubtless more frequent (137). Using interference phase contrast microscope examination of circulating red blood cells, Pearson et al (1978) found a low percentage of "pitted" red cells in 13 of 22 children splenectomized because of trauma. In five of these children a  $^{99}\text{Tc}^m$  sulfur colloid scan demonstrated multiple nodules of splenic tissue suggested to represent splenic autotransplanted tissue. This could possibly explain the somewhat lower incidence of overwhelming infections following splenectomy for trauma as compared with that after elective removal of the spleen (137). However, Spencer et al (1981) using heat damaged,  $^{99}\text{Tc}^m$ -labelled red blood cells for spleen scanning in splenectomized adults concluded that there was no difference in signs of splenic activity between patients splenectomized for trauma or hematological diseases. Since the risk of splenic autotransplantation in the latter group was minimal it favoured the opinion that activity on spleen scanning was due to the presence of accessory spleens in both groups (161). Thus, the frequency of splenic autotransplantation after splenic injury

remains to be established.

Clinically, reimplantation of splenic tissue after splenectomy is increasingly reported, obviously without serious complications (3, 18, 135). However, fatal sepsis has been reported in patients with accessory spleens or ectopic splenic tissue weighing from 4 to 92 g (74, 143, 159). It is suggested that interference with the normal circulation of blood through the spleen may be more important in determining immunological competence than the mass of splenic tissue surviving (143).

Thus, it was found that when as little as one-third was left behind after splenic resection this remnant offered almost normal resistance to pneumococci injected intravenously 16 weeks after the surgical procedure (IV). When, however, splenectomy cannot be avoided, heterotopic autotransplantation of the spleen may be a method to preserve some splenic function. Provided that the implants were of adequate size, splenic regeneration took place. In the rat, this resulted in prolonged survival time after pneumococcal challenge as compared to the asplenic rat. Whether it also would render sufficient time for effective treatment of overwhelming postsplenectomy infection requires further investigations.

#### Treatment of overwhelming pneumococcal infection in splenectomized rats (V)

Despite adequate treatment, more than 50 % mortality results when septic disease strikes the asplenic individual. This fact has not only stimulated to a search for alternatives to splenectomy, but also, if possible, to improve the therapy. Our splenectomized-rat model seems suitable for such experiments. As already mentioned, splenectomized rats are highly susceptible to pneumococci. This is clearly exemplified in Table 7. While 16 out of 18 untreated splenectomized rats died after intravenous injection of  $2 \times 10^7$  CFU of pneumococci, all of the sham-operated rats survived. Using this test system, we investigated the effect of steroids in overwhelming postsplenectomy pneumococcal sepsis.

The treated animals consisted of one group treated before and one after that the symptoms of pneumococcal sepsis were evident. The treatment was either penicillin, steroids or both given 18 or 24 hours after pneumococcal challenge and this treatment was repeated 24 hours later. Penicillin and the combination (penicillin + steroids) were effective when treatment was instituted after 18 hours, saving all the animals. However, when instituting treatment 24 hours after pneumococcal challenge, only penicillin given in combination with steroids was effective, saving 13 of 14 rats. Steroids given alone had no effect.

In man, overwhelming postsplenectomy infection may rapidly progress to shock and death within 24 hours. Postmortem examination often reveals evidence of disseminated intravascular coagulation and

sometimes the classical Waterhouse-Friederichsen syndrome is present (23, 180). Hence, immediate medical attention whenever an illness with significant fever or toxicity occurs in a splenectomized individual, is demanded. As expected, our studies in the rat showed that penicillin administration instituted early after pneumococcal challenge resulted in survival of all the animals. If, however, treatment was retarded to 24 hours after pneumococcal challenge only one fourth of the rats survived (Table 7). In agreement with these findings, Dickerman et al (1980) found therapeutic penicillin effective only when given within a certain time interval after exposure of splenectomized mice to an aerosol of pneumococci (53). These findings strongly suggest the benefit of prophylactic antibiotics in splenectomized patients (52). There is, however, a number of disadvantages with penicillin prophylaxis. Among these are patient compliance, emergence of penicillin-resistant pneumococcal strains and incomplete covering of other bacteria known to cause serious postsplenectomy infections. Despite these difficulties, many investigators advocate prophylactic penicillin in children under two years of age, since immunization against pneumococci has not yet been proved effective in these patients (62).

The use of pharmacological doses of glucocorticoids in patients with septic shock has hitherto been controversial (see 61). Schumer (1976) in a double-blind prospective randomized study reported increased survival when steroids were used in the treatment of clinical septic shock (152). Furthermore, substantial data accumulated in laboratory animals suggest that therapy with corticosteroids essentially prevents manifestation of the septic-shock syndrome (86, 142). The effect of corticosteroids has been suggested to include prevention of the early pathophysiological effects of endotoxin (142). However, although pneumococci lack endotoxin, corticosteroids proved beneficial in the present study (V). Recently, the importance of the endorphin system in the early, hyperdynamic phase of septic shock has been demonstrated, suggesting another mechanism of the steroids. Beta-lipotropin, the precursor of beta-endorphin, and ACTH originate from the same molecule. Thus, inhibition of release of ACTH using massive doses of corticosteroids, probably would also halt the release of endorphin. In the later, hypovolemic phase, complement activation results in aggregation of polymorphonuclear leucocytes starting a process leading to capillary leakage. This process is inhibited by very high concentrations of glucocorticoids. On the other hand, impairment in polymorphonuclear leucocyte migration and function by steroid administration may lead to an increased likelihood of infection outside the vascular system (for review see 61). This hypothesis of corticosteroids, counteracting the cytotoxic effects of the complement-activated polymorphonuclear cells on the vascular endothelium, may possibly explain the beneficial of combining steroids with penicillin treatment 24 hours after pneumococcal challenge in splenectomized rats registered in paper V.



Table 7. Mortality in splenectomized rats after intravenous injection of *Streptococcus pneumoniae* type 1, 200 CFU, and survival time of killed animals according to treatment (from paper V).

Treatment<sup>x</sup>

|                                                    | Steroid         |               | Penicillin  |               | Steroid + Penicillin |             | No treatment  |
|----------------------------------------------------|-----------------|---------------|-------------|---------------|----------------------|-------------|---------------|
|                                                    | 18 and 42 h     | 24 and 48 h   | 18 and 42 h | 24 and 48 h   | 18 and 42 h          | 24 and 48 h |               |
| Number of animals                                  | 10              | 14            | 10          | 14            | 10                   | 14          | 18            |
| Mortality                                          | 8               | 12            | 0           | 10            | 0                    | 1           | 16            |
| Median survival time (range) of killed animals (h) | 28.5<br>(27-36) | 39<br>(31-46) | -           | 29<br>(26-49) | -                    | 29          | 36<br>(26-60) |

<sup>x</sup>See text for specification.

## FUTURE ASPECTS

Today, it is well established that splenectomized individuals run an increased risk to contract serious infections.

Whenever presented to a splenic injury, the surgeon must adapt his treatment according to the circumstances. However, experience in both animal and man show that an attitude favouring splenic salvage should be recommended in order to reduce the risk of overwhelming postsplenectomy infections.

Increased understanding of hematological and immunological mechanisms together with the development of non-invasive methods (e.g. ultrasound and computer tomography) to exclude splenic involvement in malignant lymphoreticular diseases would probably minimize the need for diagnostic splenectomies in the future. In cases of splenectomy, vaccination against pneumococci and in the future also against Haemophilus influenzae and meningococci, should, if possible, be performed before or else after the surgical procedure. Hence, previously splenectomized patients should be traced and offered vaccination.

Increased numbers of T-lymphocytes with receptors for Fc of IgG, as demonstrated here, may indicate impaired cooperation between immunocompetent cells in splenectomized patients. Functional tests are required to further evaluate this possibility.

Treatment of overwhelming postsplenectomy infection may become more effective in the future if our results obtained in animal experiments are applicable in humans, namely that the addition of steroids to antibiotic treatment increases survival. Furthermore, methods to increase non-specific opsonization, which augments hepatic sequestration of the bacteria after splenectomy may be evaluated in animal experiments.

To summarize reevaluation of the indications for splenectomy, together with efforts to preserve the injured spleen, and the use of vaccination probably reduces the risk to contract serious post-splenectomy infections. Also, increased knowledge of normal splenic functions may result in the development of more efficient therapy against these overwhelming postsplenectomy infections.

## SUMMARY

The present work deals with mechanisms underlying the increased susceptibility to infectious disease seen after splenectomy. These infections are characterized by sudden onset, they are overwhelming and frequently resistant to therapy, leading to a mortality rate exceeding 50 %.

Most previous reports on immunological findings after splenectomy have included individuals who have been splenectomized both on hematological and traumatic indications. The misleading effect of a sometimes severe underlying disease or the presence of auto-transplanted splenic tissue was minimized in the present study by choosing patients subjected to incidental splenectomy during operation for chronic duodenal ulceration. Matched patients not splenectomized during this operation served as controls. The addition of splenectomy to the surgical procedure (highly selective vagotomy) resulted in prolonged hospital stay. The follow-up, performed almost five years after the operation, confirmed reports by others that splenectomized patients exhibit raised lymphocyte and monocyte counts. The asplenic patient also showed increased number of platelets despite the presence of normal hemoglobin concentrations. When surface markers were used to evaluate subsets of lymphocytes, the number of both T and B lymphocytes were raised. Furthermore, the number of T lymphocytes with receptors for Fc of IgG was doubled in splenectomized individuals. It has been demonstrated that this lymphocyte subset contains suppressor cells. Further functional studies are needed to evaluate this finding.

Incidentally splenectomized patients were further characterized by increased levels of IgG and complement factor C3, decreased IgM levels, but no difference in IgA and complement factor C4.

In the experimental studies it was found possible to standardize the inocula of *S. pneumoniae* type 1 for studies of pneumococcal infections in asplenic rats. An extreme susceptibility to intravenous injection of *S. pneumoniae* type 1 was demonstrated, LD<sub>100</sub> being more than 100 times less in asplenic than in control animals (II). This increased susceptibility was not altered either by variation of postsplenectomy interval or intraabdominal deposition of splenic tissue at the time of pneumococcal challenge (III). An increased survival rate was noted when splenectomized animals were challenged during a surgical procedure (III).

When evaluating alternatives to splenectomy it was found that rats, subjected to two-thirds resection of the spleen with intact circulation to the remnant, showed almost normal resistance to pneumococci 16 weeks after the surgical procedure (IV). A significant regeneration of the splenic remnant was demonstrated. Heterotopic autotransplanted splenic tissue showed signs of regeneration only when pieces and not dispersed splenic tissue was used. Thus, dis-

persed splenic tissue injected subcutaneously, intramuscularly or retroperitoneally, showed a negligible regeneration. Consequently, 16 weeks after splenic autotransplantation such animals demonstrated identical susceptibility to pneumococci as asplenic animals. When, however, the spleen was divided into 12 equal pieces, implanted subcutaneously or sutured to the greater omentum, significant regeneration and prolonged survival time after pneumococcal challenge were registered 16 weeks after the surgical procedures. Regenerated splenic tissue showed preserved microarchitecture compared to that of the normal spleen. It was concluded that two-thirds resection of the spleen was more efficient than autotransplantation of the spleen with respect to protection against intravenously injected pneumococci (IV).

It is characteristic to postsplenectomy infections with S. pneumoniae that the mortality rate exceeds 50 %. Treatment of asplenic rats with penicillin after pneumococcal challenge was efficient if given within 18 hours, but if retarded to 24 hours after intravenous injection of the bacteria penicillin was effective only if combined with pharmacological doses of steroids (V).

Caution is required when applying findings in an animal model to man. There are, however, similarities indicating that results obtained in the splenectomized-rat model may further elucidate the immuno(in)competence after splenectomy in man.



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